

We claim:

1. A method of producing insulinotropic GLP-1(7-36) polypeptide and/or GLP-1 analogs comprising:

(a) introducing two individual restriction endonuclease cleavage sites capable of forming a hybrid site to two terminals of a gene which may encode the GLP-1(7-36) polypeptide or GLP-1 analogs;

(b) ligating cohesive ends to form a hybrid site after digestion with restriction endonucleases, and cloning into a vector N copies of a resulting series-linked GLP-1(7-36) gene, GLP-1 analog gene, or combination genes encoding GLP-1(7-36) polypeptide or GLP-1 analogs, wherein N is an integer from 1 to 32;

(c) transforming a vector containing the series-linked gene into a host cell;

(d) expressing into the host cell a fusion protein comprising N copies of the series-linked GLP-1(7-36) polypeptide, GLP-1 analog or combination thereof, but without any carrier protein;

(e) cleaving the fusion protein; and

(f) separating and purifying the GLP-1 (7-36) polypeptides and/or GLP-1 analogs.

2. The method according to claim 1 wherein the two restriction endonucleases capable of forming a hybrid site are Bgl II and BamH I.

3. The method according to claim 1 wherein the two restriction endonucleases capable of forming a hybrid site are Sal I and XhoI.

4. The method according to claim 1 in which said vector contains N copies of the series-linked gene, wherein N is an integer from 2 to 32.

5. The method according to claim 4 in which the said vector contains N copies of the series-linked gene, wherein N is an integer from 8 to 32.

6. The method according to claim 5 in which the said vector contains N copies of the series-linked gene, wherein N is 16.
7. The method according to claim 5 in which the said vector contains N copies of the series-linked gene, wherein N is 32.
8. The method according to claim 6 wherein said vector is the one contained in the deposit of CGMCC Accession No.0599.
9. The method according to claim 1 in which said host cell may express a fusion protein containing N copies of a polypeptide, wherein N is an integer from 1 to 32.
10. The method according to claim 9 in which said host cell may express a fusion protein containing N copies of a polypeptide, wherein N is an integer from 8 to 32.
11. The method according to claim 10 in which said host cell can express a fusion protein containing N copies of a polypeptide, wherein N is 16.
12. The method according to claim 10 in which said host cell can express a fusion protein containing N copies of a polypeptide, wherein N is 32.
13. The method according to claim 9 wherein said host cell is a prokaryotic cell.
14. The method according to claim 13 wherein said host cell is *Escherichia coli* JM103, JM109, HB101, or DH5 $\alpha$  or C600.
15. The method according to claim 14 wherein said host cell is the one contained in CGMCC Deposit No. 0599.

16. The method according to claim 1 wherein said protease used to cleave the fusion protein is Clostrispan or Trypsin.

17. The GLP-1 (7-36) polypeptide and/or GLP-1 analog produced according to the method of claim 1.

18. The GLP-1 (7-36) polypeptide according to claim 17, having an amino acid sequence of which is shown in Formula I:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-  
Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-OH

Formula I

19. A method of producing an expression vector comprising multiple tandem copies of a gene encoding a desired polypeptide comprising the steps of:

(a) constructing a vector comprising the gene and four individual restriction enzyme sites A - D in a relative order A - C - gene - B - D, wherein restriction enzyme sites C and B are capable of forming a hybrid site lacking restriction enzyme sites C and B;

(b) digesting an aliquot of the vector comprising the gene with endonucleases C and D and isolating a resulting double digested gene fragment;

(c) digesting a second aliquot of the vector with endonucleases B and D and isolating a resulting double digested vector including the gene;

(d) ligating the double digested gene fragment and the double digested vector comprising the gene to form a vector comprising N tandem copies of the gene linked by the hybrid site lacking restriction enzyme sites C and B; and

(e) repeating steps b - d, wherein each repeating series of steps begins with the vector product of step d such that the N tandem copies of the gene double with each series.

20. The method of claim 19, wherein the gene encodes one or more additional N-terminal amino acids selected from the group consisting of: Met; Arg; Met-Arg; Met-Met-Arg; Asp-Asp-Asp-Asp-Lys; and combinations thereof.
21. The method of claim 19, wherein the polypeptide is insulinotropic.
22. The method of claim 21, wherein the insulinotropic polypeptide selected from the group consisting of: GLP-1(7-36); GLP-1 analogs; and exendin-4 analogs.
23. The method of claim 22, wherein N is an integer from 2 to 32.
24. The method according to claim 23, wherein N is an integer from 8 to 32.
25. The method of claim 19, wherein restriction endonucleases sites C and B capable of forming a hybrid site are Bgl II and BamH I.
26. The method of claim 19, wherein restriction endonucleases sites C and B capable of forming a hybrid site are Sal I and Xho I.
27. A method of producing an insulinotropic polypeptide comprising:
  - (a) expressing into a host cell a fusion protein comprising 2 - 32 tandem copies of the insulinotropic polypeptide, wherein each copy comprises a cleavable N-terminal Arg or cleavable spacer;
  - (b) isolating the fusion protein from the host cells;
  - (c) cleaving the fusion protein at the cleavable N-terminal Arg or cleavable spacer; and
  - (d) separating and purifying the insulinotropic polypeptide.

28. The method of claim 27, wherein the fusion protein is cleaved by treatment with a compound selected from the group consisting of: cyanogen bromide, alkaline proteases, enterokinases, endopeptidases, and combinations thereof.
29. The method of claim 28, wherein the alkaline protease is trypsin and internal lysine groups are acetylated prior to trypsin treatment.
30. The method of claim 29, wherein the internal lysine groups are acetylated by treatment with an anhydride followed by deprotection after trypsin treatment.
31. The method of claim 30, wherein the anhydride is selected from the group consisting of: acetic anhydride; maleic anhydride; citraconic anhydride, and 3, 4, 5, 6-tetrahydrophthalic anhydride.
32. The method of claim 27, wherein the insulinotropic polypeptide is selected from the group consisting of: GLP-1(7-36) (SEQ ID NO:1), GLP-1(7-36)-NH<sub>2</sub> (SEQ ID NO:2), Gly<sup>8</sup>-GLP-1(7-36) (SEQ ID NO:4), Val<sup>8</sup>-GLP-1(7-36) (SEQ ID NO:5), Asp<sup>11</sup>-GLP-1(7-36) (SEQ ID NO:6), Ala<sup>16</sup>-GLP-1(7-36) (SEQ ID NO:7), Glu<sup>22</sup>-GLP-1(7-36) (SEQ ID NO:8), His<sup>23</sup>-GLP-1(7-36) (SEQ ID NO:9), Glu<sup>24</sup>-GLP-1(7-36) (SEQ ID NO:10), Trp<sup>26</sup>-GLP-1(7-36) (SEQ ID NO:11), Ala<sup>27</sup>-GLP-1(7-36) (SEQ ID NO:12), Glu<sup>30</sup>-GLP-1(7-36) (SEQ ID NO:13), Asp<sup>33</sup>-GLP-1(7-36) (SEQ ID NO:14), Glu<sup>34</sup>-GLP-1(7-36) (SEQ ID NO:15), Thr<sup>35</sup>-GLP-1(7-36) (SEQ ID NO:16), Gly<sup>8</sup>-Glu<sup>24</sup>-GLP-1(7-36) (SEQ ID NO:17), Leu<sup>8</sup>-Ala<sup>33</sup>-GLP-1(7-36) (SEQ ID NO:18), and exendin-4 analogs.
33. The method of claim 32, wherein the insulinotropic polypeptide is GLP-1 (7-36) (SEQ ID NO 1) and the cleavage spacer is an N-terminal Arg.

34. The method of claim 32, where the insulinotropic polypeptide is GLP-1 (7-36) (SEQ ID NO 1) and each GLP-1 copy is preceded by an N-terminal Met-Arg.

35. The method of claim 34, wherein the isolated fusion protein is treated with cyanogen bromide followed by cleavage with clostripain protease.

36. The method of claim 27, wherein the fusion protein has a coding sequence selected from the group consisting of: SEQ ID NO: 29 and SEQ ID NO:30.